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Short Communication: Energetics of glucoamylase-catalyzed hydrolysis of commercial sago starch

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Abstract. Glucoamylase (EC 3.2.1.3) from *Aspergillus niger* was used to hydrolyze commercial sago starch into reducing sugars. The energetics of the hydrolysis was studied at optimal substrate concentration, enzyme amount and pH for the process. The ΔH and ΔS estimated via van't Hoff analysis were 7.9 J.mole^{-1} and 6.2 J.mole^{-1}.K^{-1}, respectively. The results indicated that the hydrolysis process is an endothermic reaction and spontaneous within the temperature range tested. It is calculated that 26.3 J.mole^{-1} of activation energy, E_a is required for the enzymatic conversion of sago starch into reducing sugars. The spontaneous reaction with relatively low activation energy supported the viability of glucoamylase-catalyzed hydrolysis of sago starch.

Keywords: Energetics , Glucoamylase, Reducing sugars, Sago starch.

INTRODUCTION

Starch production by sago palm is concentrated in the Asia Pacific region and South East Asia (Singhal *et al.*, 2008). At the end of 20th century, it is estimated that about 60 million tons of sago starch, extracted from sago palms, are produced per annum in South-East Asia alone (Wang *et al.*, 1996). In terms of yield, sago produces a higher amount of starch, at 2 to 3 tons of starch per hectare per year as compared to crops like cassava (2 tons) and maize (1 ton) (Stanton, 1993). The world's biggest exporter of sago, Malaysia, exports 25,000 to 40,000 tons of sago products annually (Singhal *et al.*, 2008). Due to its abundance, sago may be utilized for the production of fermentable sugars. This can be accomplished economically on a large scale with the use of starch-saccharifying enzymes. Adinarayan and Suren (2005) have reported that processes using starch-saccharifying enzymes have replaced the conventional method of starch hydrolysis using acid. These enzymes account for approximately a 15% share in the global enzyme market. One such enzyme is glucoamylase (EC 3.2.1.3), which hydrolyzes starch into reducing sugars such as glucose and maltose. In this study, glucoamylase from *Aspergillus niger* was used to hydrolyze commercial sago starch into reducing sugars. The sugars from sago starch can be used as feedstocks in the fermentation industries and also for the production of high-fructose syrup (Suraini, 2002).

It is well established that an increase in temperature influences enzyme-mediated catalysis by increasing the rate constant of the reaction until a maximum is reached, after which a steep decline is observed with further increase in temperature. This decline in rate constant is due to excessive thermal effect at elevated temperatures affecting the three-dimensional conformation of the enzyme resulting in loss of catalytic activity (Cornish-Bowden, 1972) and enzyme denaturation. Therefore, it is important to understand the effect of temperature on enzyme-catalyzed reactions.

Thermodynamic investigation of enzyme-catalyzed reactions seeks to characterize the quantitative relationships between heat and other forms of energy and/or analyze the energy changes accompanying material transformations. It is used to predict the direction in which forces will be acting in the systems and the magnitude of these driving forces. It also provides a tool for understanding and predicting the behavior of systems in terms of their energetics, which is useful in designing a rational process. Hence, in order to understand the macroscopic thermodynamic behavior of commercial sago starch hydrolysis by glucoamylase, parameters such as enthalpy change (ΔH), entropy change (ΔS), Gibbs free energy change (ΔG) and activation energy (E_a) were determined from the experimental data.

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MATERIALS AND METHODS

Substrate The sago starch used in this study was isolated from the sago palm, *Metroxylon sagu* and was obtained from commercial producer, Wah Chang International, Malaysia. The starch was dried to a constant weight at 60°C before use. The chemical composition of sago starch (dry weight basis, %) is as follows: amylase 27; amylopection 73; lipids 0.1; protein (nitrogen content x 6.25) 0.1; ash 0.2; phosphorus 0.02 (Swinkels, 1985).

Enzyme Glucoamylase (EC 3.2.1.3) from *Aspergillus niger* was purchased from Sigma-Aldrich (www.sigmaaldrich.com). The specific activity of the enzyme preparation, was 31.2 U.mg^{-1} protein, according to the manufacturer. The enzyme concentration (expressed as U.ml^{-1}) used for the study was calculated based on this information.

Thermodynamics of sago starch hydrolysis by glucoamylase Optimal conditions for the glucoamylase-catalyzed hydrolysis of commercial sago starch in a stirred tank reactor (STR) have been determined using statistical experimental design in our previous study (Wee *et al.*, 2011). Hydrolysis of sago starch to reducing sugars was found to be optimal at a pH of 4.5 and an enzyme-to-sago starch ratio of 200 U per gram of starch (or equivalent to 0.2 U.ml-1 in this study). In this study, the temperature was varied from 30 to 60°C. The experiments were performed in test tubes and repeated three times. The reactions progressed until equilibrium and the amount of product, i.e. reducing sugars, was determined using the dinitrosalicylic (DNS) acid method (Miller, 1959). Concentration of the reducing sugars was routinely measured spectrophotometrically at 575 nm. The absorbance was converted to concentration using a calibration curve that fitted the following equation:

$$C_{rs} = 769 Abs_{575} \quad (1)$$

where C_{rs} was the concentration of the reducing sugars in μg.ml^{-1} and A_{575} was the absorbance of the solution at 575 nm. Equation (1) had a regression coefficient of 0.996 and applied over a concentration range from 0 to 700 μg.ml^{-1} glucose standard solution. A control blank containing an initial concentration of un-reacted starch and heat-deactivated glucoamylase was prepared to correct for the baseline reading in the sample measurement.

Calculation of apparent thermodynamics parameters The changes in enthalpy ΔH (J.mol^{-1}) and entropy ΔS (J.mol^{-1}.K^{-1}) of the reaction were determined from the slope and intercept of a van't Hoff plot, respectively:

$$\log K_{eq} = \frac{\Delta H}{2.3RT} + \frac{\Delta S}{2.3R} \quad (2)$$

The apparent K_{eq}^{app} was calculated as follows:

$$K_{eq}^{app} = \frac{[D_{eq}]}{[D_0 - D_{eq}]} \quad (3)$$

The concentrations D_0 and D_{eq} represent the initial reactant added and reducing sugars (product) at equilibrium, respectively. The assumptions implicit in van't Hoff analysis (i.e. a closed system at a constant pressure and volume) were applicable to the hydrolysis reaction system studied.

The apparent Gibbs energy change ΔG (J.mole^{-1}) of the reaction at constant temperature and pressure was calculated as follows:

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

Calculation of apparent rate constant, **k'** The apparent rate constant, *k'* (mmoles sugar.U^{-1}.min^{-1}) was calculated using the relationship:

$$v = k'[E]_m \quad (5)$$

where v is the initial velocity (mmoles.L^{-1}.min^{-1}) and $[E]_m$ is measured enzyme activity (U.ml^{-1}). To determine the v, a polynomial model representing progress curve for product formation was generated using the least-square method of data fitting. v is the maximum tangent from the origin following differentiation.

Calculation of activation energy, **E_a** The activation energy E_a of the reaction was calculated using an Arrhenius plot. Thus, the apparent rate constant *k'* values measured at various temperatures T were plotted in the following form and the slope was calculated as E_a:

$$\log k = \log A - \frac{E_a}{2.3R}\frac{1}{T} \quad (6)$$

where A is Arrhenius parameter; R is universal gas constant (8.314 J.mol^{-1}.K^{-1}).

RESULTS AND DICUSSION

Reaction energetics at optimal conditions: the effect of temperature The sago starch hydrolysis curves at different temperatures from 30 to 60°C are shown in Figure 1. The time progress curves were used to determine the apparent first-order rate constant, *k'* (equivalent to initial reaction velocity, v divided by measured enzyme activity, $[E]_m$ as shown in Table 1). It is clear that the hydrolysis of sago starch to reducing sugars was strongly enhanced with increasing temperature, due to the fact that an increase in temperature imparts more kinetic energy to the reactant molecules and leads to more productive overcoming of the energy barrier per unit time. Therefore, a higher product yields were obtained at 60°C than at lower temperatures. The glucoamyl-

Table 1. Progress curve equation and initial reaction velocity, *v* at different temperatures.

Temperature, *T* (°C)	Progress curve equation, *p*	$\frac{dp}{dt}$	Initial reaction velocity, *v* (mmoles.L^{-1}.min^{-1})	Apparent rate constant, *k'* (mole.U^{-1}.min^{-1})
60	p = -0.0058 t^2 + 0.4111 t	-0.0116 t + 0.4111	0.41	2.05 x 10^{-6}
50	p = -0.0037 t^2 + 0.3020 t	-0.0074 t + 0.3020	0.30	1.50 x 10^{-6}
40	p = -0.0026 t^2 + 0.2245 t	-0.0052 t + 0.2245	0.22	1.10 x 10^{-6}
30	p = -0.0015 t^2 + 0.1619 t	-0.0030 t + 0.1619	0.16	0.80 x 10^{-6}

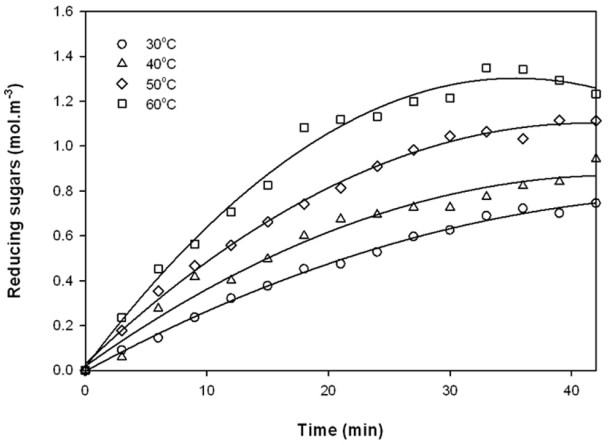

Figure 1. Effect of temperatures (30 to 60°C) on the hydrolysis of sago starch. ([*S*] 2.0 g.L^{-1}, [*E*] 0.2 U.ml-1 and pH 4.5).

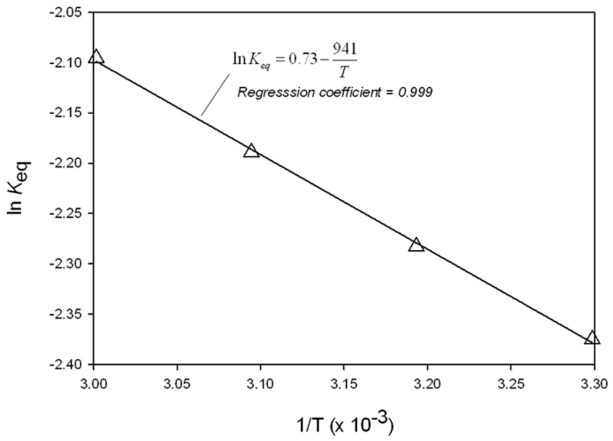

Figure 2. van't Hoff plot for sago starch hydrolysis by glucoamylase.

ase activity performed satisfactorily at this temperature range since 40 to 60°C is considered optimum for this enzyme (Carlos *et al*, 2006).

Calculation of apparent equilibrium constant, K_{eq} The calculated values of apparent equilibrium constant, K_{eq} at different temperatures were tabulated in Table 2. Once the value of K_{eq} has been obtained, it can be used to construct the van't Hoff plot and calculate the enthalpy change (ΔH), entropy change (ΔS) and Gibbs free energy change (ΔG).

Table 2. Apparent equilibrium constant, K_{eq}^{app} different temperatures.

T (K)	$[P]_{eq}$	$[S]_{eq}$	K_{eq}^{app}	K_{eq}^{app} ln
303	0.170	1.830	0.093	-2.378
313	0.185	1.815	0.102	-2.283
323	0.201	1.799	0.112	-2.189
333	0.219	1.781	0.123	-2.096

Calculation of energetics parameters, ΔH and ΔS Figure 2 shows the van't Hoff analysis. The ΔH and ΔS calculated from graph were 7.9 J.mole^{-1} and 6.2 J.mole^{-1}.K^{-1}, respectively. Both values obtained were based on assumptions implicit in van't Hoff analysis applied to the hydrolysis reaction i.e. in a closed system at a constant pressure and volume.

The positive value of enthalpy, 7.9 J.mole^{-1} ($\Delta H > 0$) indicates the process is endothermic and that heat is absorbed from the surroundings. However, the positive sign says nothing about whether a process occurs either spontaneously or not. Therefore, by evaluating the value of entropy, ΔS helps to predict and determine whether a process is spontaneous or otherwise (Silberberg, 2006). The positive sign of entropy, 6.2 J.mole^{-1}.K^{-1} ($\Delta S > 0$) confirmed that the hydrolysis reaction for sago starch occurred spontaneously.

Calculation of Gibbs free energy change, ΔG The values of ΔG, (ΔG–ΔG^o) and ln K_{eq} were tabulated in Table 3 at different temperatures. The ΔG is defined as being equal to the enthalpy of a system minus the product of entropy and temperature as shown in Equation (4). Both positive signs of ΔH and ΔS have influence on value of ΔG, where ΔG is positive when at low temperature and is negative at high temperature.

The negative values of ΔG ($\Delta G < 0$) in Table 3 for all range of temperatures (30–60°C) indicate that the hydrolysis reaction of sago starch by glucoamylase is spontaneous and occurred in the forward direction at all tested temperatures. The values of (ΔG–ΔG^o) indicate the difference between the energy changes during the reaction under the conditions tested and the energy change that would occur if the reaction took place under standard conditions (1 atm, 298.15 K, ideal solution).

Table 3. Gibbs free energy change (Δ*G*) for sago starch hydrolysis by glucoamylase.

T (°K)	ln K_{eq}	Δ*G* (J.mol^{-1})	(Δ*G*–Δ*G*°) (J.mol^{-1})
303	-2.378	-1857	-5993
313	-2.283	-1919	-5944
323	-2.189	-1980	-5883
333	-2.096	-2042	-5807

Calculation of activation energy, E_a The resulting *k'* from Table 1.0 was then replotted against temperature using an integrated Arrhenius equation (6) as shown in Figure 3. The E_a value calculated from the slope of the resulting plot was 26.3 J.mole^{-1}. This value obtained was consistent with activation energy values for enzyme-catalyzed reaction that generally range from 16 to 84 J.mol^{-1} (Shuler and Kargi, 1992). This is a minimum activation energy required to convert the sago starch to the form of reducing sugars when the reaction is catalyzed by glucoamylase.

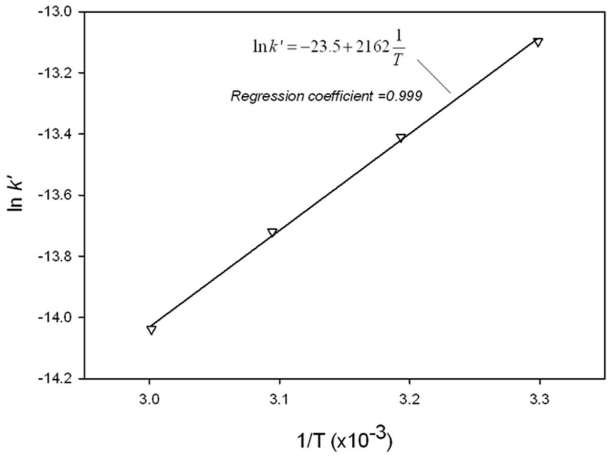

Figure 3. Arrhenius plot for sago starch hydrolysis by glucoamylase.

CONCLUSION

Glucoamylase-catalyzed hydrolysis of commercial sago starch into reducing sugars is a spontaneous process with relatively low activation energy. Hence, this study provides a strong support for the application and process viability of enzyme-mediated hydrolysis of starch in the industrial production of sugars.

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